



## Defatted cashew nut shell starch as renewable polymeric material: Isolation and characterization

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### ABSTRACT

Starch attracts public attention as a replacement of fossil fuel in polymer industries because it is renewable, biodegradable and nontoxic. In this study, the isolation of starch from defatted cashew nut shell (CNS) using wet milling was reported. A product that contains 85.01 wt.% starch was recovered from the defatted CNS. Various analyses were performed on the starch to characterize its physicochemical properties. It was found that the starch obtained possesses high amylopectin content (75.35 wt.%), which supports the results of thermal analysis that proved the high crystallinity of starch. Morphological study of the starch showed that bonded resins were found attached to the starch granules. Due to high crystallinity, the presence of bonded resins and low cost, starch from defatted CNS can be considered as a prospective renewable material in polymer industries, with potential to compete with current feedstock such as potato and corn.

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### 1. Introduction

The use of fossil fuels such as naphtha and natural gas for producing plastic resins accounts for about 4–5% of the world's oil consumption, with the increasing demand in the future. A challenge comes from the society to reduce the exploitation of fossil fuel and to protect the climate through the reduction of CO<sub>2</sub> released, as well as to preserve the environment from the harmful effects of the indiscriminate plastic disposal. These issues, especially the disposal of plastic wastes in the environment, stimulated a demand for harmless, eco-friendly and biodegradable materials. This then evolved to the adoption of the recycling concept through mechanical recovery and composting of wastes or energy production by plastic incineration, which directly contribute towards the reduction of the consumption of fossil raw materials in industry<sup>1</sup>. However, the focus soon began to shift to the plastic production using the renewable materials as a replacement of the petrochemical substances.

Starch, cellulose, sugar, vegetable oil, and wood are the most frequently used natural and renewable raw materials in the direct manufacturing of biodegradable plastics (hereafter will be called as bioplastics) or its bio-intermediaries. Queiroz and Collares-

Queiroz (2009) reported that starch-based bioplastic production covered around 20% of the total world production of bioplastic. The properties of natural polymers derived from starch, which are biodegradable, usually can be modified by blending with polycaprolactone (PCL), polyvinyl alcohols (PVOH), and other chemicals.

Starch serves as the major source of polysaccharide in plants that provides the bulk nutrient and energy source in human diet (Galliard, 1987; Shelton & Lee, 2000). It finds wide applications not only in food but also in pharmaceutical, biomedical and polymer industries because of its biocompatibility, biodegradability, non-toxicity, and abundant sources. Naturally, starch is semicrystalline substance with varying levels of crystallinity. The crystallinity is associated with the amylopectin compound, while the amorphous regions mainly represent amylose (Zobel, 1988a,b). Behavior of starch in aqueous systems depends on the physical and chemical characteristics of the starch granules, such as mean granule size, granule size distribution, amylose/amylopectin ratio and mineral content (Madsen & Christensen, 1996). Interest in new value-added starch products to the industry has resulted in many studies being carried out on the morphological, rheological, thermal and textural properties of starches. Identification of native starch sources is required for determine its desired functionality and unique properties. Cashew (*Anacardium occidentale* L.) is an indigenous tree of Brazil and grows well in some tropical countries in Asia and Africa, such as Mozambique, Vietnam, Sri Lanka, Malaysia, India and Indonesia (Assunção & Mercadante, 2003; Michodjehoun-Mestres, Souquet, Fulcrand, Bouchut, Reynes & Brillouet, 2009;

Abbreviation: CNS, cashew nut-shell.

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Shobha, Krishnaswamy, & Ravindranath, 1992). The nut is considered as the most important part in international market due to its widespread acceptance and demand (MacLeod & Troconis, 1982; Maia, Andrade, & Zoghbi, 2000). Cashew nut comprises the kernel, which is edible and nutritionally valuable, and the shell, which is known as inedible by-product of cashew nut production and a well-known source for unsaturated long-chain phenols, such as anacardic acids, anacardols, cardols and their isomers. However after the removal of the phenols and lipids, defatted cashew nut-shell (CNS) may cause an environmental problem if it is not handled properly. Utilizing the defatted CNS as a starch source for bioplastic production is a way to reduce the waste disposal from the cashew nut production. Moreover, the use of defatted CNS, due to its existence as by-product, may provide the cost reduction of raw material in the bioplastic production, which is currently dominated by higher price feedstock such as potato and corn.

There has been no report published on the isolation of starch from defatted CNS and to date, no data is available on the starch properties of the defatted CNS. This study focused on the isolation and characterization of starch from defatted CNS. The feasibility of this starch as a renewable material for commercial application such as starch-based bioplastic production was also observed.

## 2. Materials and methods

### 2.1. Materials

CNSs were obtained from the waste of cashew (variety *Venguria-4*) nut production in a factory in Solo, Indonesia. They were grounded, sieved, and stored at  $-4^{\circ}\text{C}$  to minimize the degradation of its compounds. Defatting of CNSs was carried out using methanol at  $65^{\circ}\text{C}$  for 10 h followed by *n*-hexane at  $69^{\circ}\text{C}$  for another 10 h in a soxhlet extractor.

The two solvents used for defatting CNSs were of HPLC grade. *n*-Hexane (95% purity) was purchased from Tedia (OH, USA) while methanol (99.5% purity) was obtained from Echo Chemical (Miao Li, Taiwan). Standards for glucose, amylose from potato starch and amylopectin from maize were purchased from Sigma–Aldrich (St. Louis, MO). Enzymes for starch analysis, namely  $\alpha$ -amylase (EC 3.2.1.1), protease (EC 3.4.23.18) and amyloglucosidase (EC 3.2.1.3) were also obtained from Sigma–Aldrich.

### 2.2. Purification of starch from defatted CNS

The isolation of starch from defatted CNS was carried out using the modified method of Fabian, Ayucitra, Ismadji, and Ju (2011). CNS (10 g) was soaked in water with a CNS to water ratio of 1–5 (w/w) for 3 h at  $30^{\circ}\text{C}$ . The mixture was blended for 5 min and screened using a 60-mesh sieve. The residue was re-blended with 50 ml 70% ethanol for 5 min, passed through a 60-mesh sieve and then the residue was re-blended with 50 ml 0.1 M NaOH for another 5 min, and screened using a 60-mesh sieve. The filtrates obtained were combined and centrifuged at  $11,000 \times g$  for 15 min. The supernatant was then decanted carefully and the residue was re-slurried with 100 ml of water, re-filtered twice through a 200 mesh screen and a Whatman analytical grade no.5 filter paper with  $2.5 \mu\text{m}$  pore size and then washed successively with 0.1 M NaOH and deionized water. The residue restrained at the filter paper was dried by using a freeze drier (Labconco Free Zone 2.5 Benchtop freeze dry system model 7670520, Kansas City, MO). The dried starch was kept at  $-5^{\circ}\text{C}$  prior to analysis. All purification and analysis experiments were done at least in duplicates.

### 2.3. Starch analysis

#### 2.3.1. Total starch analysis

Total starch content was analyzed using the modified method of AOAC Official Method 996-11 (1996). In spite of using glucose oxidase–peroxidase–aminoantipyrine buffer mixture as the reagent mentioned in the official method, 3,5-dinitrosalicylic acid or popularly known as DNS was chosen since this reagent is unaffected by the interference of xylose presence in the sample and has a good stability of color development.

#### 2.3.2. Protein, ash and total dietary fiber analysis (TDF)

The protein and ash content were determined by AOCS Official Methods Ba 4a-38 (1997) and Ba 52-49 (1997), respectively. Total dietary fiber was analyzed by using the modified method of Prosky, Asp, Scheweizer, de Vries, and Furda (1988) which is discussed by Fabian et al. (2011). The TDF content was measured as the weight of residue less its protein and ash.

#### 2.3.3. Total amylose content analysis

Amylose content in starch was determined by using the method of Sadasivam and Manickam (1996). Total amylose content was analyzed using a Jasco UV–vis spectrophotometer (UV–V 550) at 590 nm.

#### 2.3.4. Swelling and solubility

The study of swelling and solubility were adapted from Singh, Okadome, Toyoshima, Isobe, and Ohtsubo (2000). Defatted CNS (0.5 g) was mixed with 20 ml water and the mixture was heated from  $30^{\circ}\text{C}$  to  $90^{\circ}\text{C}$  in 30 min. The sample–water mixture was then weighed and more water was added until the weight of the mixture reached 25 g. Centrifugation at  $11,000 \times g$  for 15 min was performed to separate the solid residue and the supernatant. Swelling power was determined by using the following formula:

$$\text{Swelling power} = \frac{\text{Wet residue weight (g)} - \text{dry defatted CNS (g)}}{\text{dry defatted CNS (g)}}$$

For determining the solubility of starch, about 10 ml of supernatant was freeze dried using a freeze drier (Labconco Free Zone 2.5 Benchtop freeze dry system model 7670520, Kansas City, MO). The dried soluble starch was then weighed and the solubility was calculated by the equation proposed by Singh et al. (2000)

$$\text{Solubility\%} = \frac{\text{dry residue starch}}{\text{dry defatted CNS}} \times \frac{25 \text{ ml}}{10 \text{ ml}} \times 100$$

#### 2.3.5. Thermal analysis

Retrogradation property of defatted CNS starch was analyzed using DSC Jade (Perkin Elmer, Japan). About 6 mg defatted CNS starch was weighed and put in a  $40 \mu\text{l}$  aluminum pan (TA instrument, USA). The sample was sealed and kept for 1 h at  $30^{\circ}\text{C}$  prior to analysis. The DSC was calibrated by indium and an empty aluminum pan was used as the reference. Sample pans were heated from  $25^{\circ}\text{C}$  to  $200^{\circ}\text{C}$  at  $10^{\circ}\text{C}/\text{min}$ . The onset temperature ( $T_o$ ), peak temperature ( $T_p$ ) and enthalpy of gelatinization ( $\Delta H_{\text{gel}}$ ) were calculated automatically by Pyris thermal data analysis software. The sample was then cooled down to  $4^{\circ}\text{C}$  for 7 days and re-heated from  $25^{\circ}\text{C}$  to  $200^{\circ}\text{C}$  at  $10^{\circ}\text{C}/\text{min}$  to measure the retrogradation. The enthalpy of retrogradation ( $\Delta H_{\text{ret}}$ ) was determined by the software and the percentage of retrogradation was calculated using the following equation:

$$\% \text{retrogradation} = \frac{\Delta H_{\text{ret}}}{\Delta H_{\text{gel}}}$$

On the other hand, the thermal stability of starch was studied using Diamond TG/DTA instrument (Perkin Elmer, Japan). A 6 mg

**Table 1**  
Composition of defatted CNS starch.

Component	wt.%
Starch	85.01
Protein	0.93
Total dietary fiber	3.74
Ash	4.23
Others	6.09

sample was placed in a platinum pan and heated from 30 °C to 900 °C at 10 °C/min to monitor the temperature at which decomposition occurred. During the entire process, nitrogen at 3.7 bar was delivered through the system containing the sample at 20 ml/min.

### 2.3.6. Scanning electron microscopy (SEM)

A Cambridge SEM S-360 with an accelerating voltage of 20 kV was used to take micrographs of the defatted CNS starch. The starch grains were scattered on the surface of a double-sided tape which is attached to a stub and coated with gold.

## 3. Results and discussion

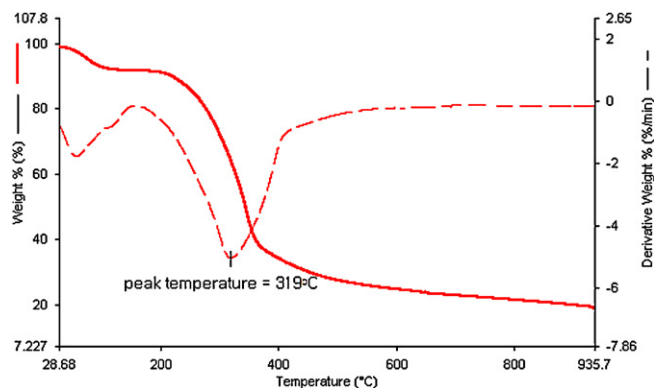
### 3.1. Starch purity

Five components were observed in the initial composition analysis of defatted CNS. They are starch, protein, fiber, ash and other impurities with content of 15.98 wt.%, 26.17 wt.%, 27.09 wt.%, 22.77 wt.% and 7.99 wt.%, respectively. During starch isolation, 3 extracting agents, viz. water, 70% ethanol and 0.1 M NaOH were used to extract starch from the defatted CNS. However since it is difficult to separate protein from starch due to the complex bonding of protein to amylose and amylopectin, water and 0.1 M NaOH were used as washing agents to reduce protein content to as low as possible. Starch isolated from defatted CNS contains impurities such as protein and dietary fiber as shown in Table 1. From the results of starch morphology study which will be presented in Section 3.4, the other impurities may be polymeric material bonded to starch granules.

### 3.2. Physicochemical properties of starch

Starch is the major polysaccharide source in plants and presents naturally in the form of granules. Amylose and amylopectin are two major components comprising starch and play major role in determining the level of starch crystallinity. Amylose content is related to the amorphous state of starch while amylopectin is correlated to its crystallinity. Based on its amylose content, starch can be classified into 3 groups, namely amylase-rich starch (amylose content > 30 wt.%), moderate amylose starch (amylose content 10–30 wt.%) and waxy starch (amylose content < 10 wt.%). In this study, defatted CNS starch with moderate amylose content of 24.65 wt.% was obtained. Phosphate, which is one of the non-carbohydrate constituents in starch (mostly as phospholipid), was not detected in this study due to the defatting process employed prior to starch purification. Therefore, it is assumed that CNS starch may contain only amylose and amylopectin as the major compounds, with an amylopectin content of 75.35 wt.%.

According to Leach (1965), swelling and solubility are two of the important properties of starch in commercial application. Swelling and solubility of starch granules occur when starch is heated in excess water and its crystalline structure is disrupted. Water molecules become linked by hydrogen bonding to the exposed hydroxyl groups of amylose and amylopectin, which causes the swelling and solubility of granules. Madsen and Christensen (1996) reported that crystallinity of starch determines the behavior of



**Fig. 1.** Thermogravimetric curves of defatted CNS starch. Weight loss curve (—). Derivative weight loss curve (---).

starch paste which is an important parameter in starch-based polymer industries. The branched polymer, amylopectin, swells to a greater extent along with protein while amylose plays a role in restricting swelling and increase starch solubility. However, despite having high concentration of amylopectin, it can be seen in Table 2 that the swelling power of defatted CNS starch is 5.12 g/g, which is much lower than that of corn starch with a lower amylopectin content (~51 wt.%) (Singh, Singh, Kaur, Sodhi, & Gill, 2003). This result may be attributed to the presence of resins in starch as mentioned in Section 3.4. Wang, Liu, and Sun (2003) reported that resins bonded to starch granule will reduce the swelling power of starch.

As also shown in Table 2, solubility of defatted CNS starch was found to be 48.48%, two times higher than that of commercial corn starch which has similar amylase content. According to Fabian et al. (2011), this phenomena was likely caused by the processes it underwent such as defatting and soaking in water during starch purification.

### 3.3. Thermal properties of starch

Fig. 1 shows thermal decomposition profiles of the defatted CNS starch. The first thermal degradation occurred from 28.68 to 123 °C. It was likely due to the dehydration of sample. The second thermal degradation, which was started at 174 °C and ended at 370 °C, caused a significant decrease in sample weight. At 319 °C, major decomposition happened which resulted in a sample weight loss of 47%. This was probably caused by the degradation of glucose ring in the polymer composing the starch (amylose and/or amylopectin). Liu, Xie, Yu, Chen, and Li (2009) stated that glucose ring in the starch started to decompose at 220 °C and degraded rapidly at temperature above 260 °C. After this second degradation, a constant decomposition occurred from 370 °C to 639 °C. These results suggested that if defatted CNS starch is to be used in application, any processing which requires temperature higher than 174 °C should be avoided.

In addition to decomposition, gelatinization and retrogradation are also important thermal properties of starch. As mentioned in Section 3.2, starch is composed of amylose, which is related to the amorphous state of starch, and amylopectin which is associated with crystallinity of starch. Initially, gelatinization occurs in its amorphous regions of starch because hydrogen bonding is rapidly weakened in these areas (Kim, Wiesenborn, Orr, & Grant, 1995). However, according to Flipse, Keetels, Jacobson, and Visser (1996), amylopectin is the one that plays a major role in starch granule crystallinity. Higher amylopectin content results in an increase of structural stability, resistance towards gelatinization and energy for starting starch gelatinization, thus leads to an

**Table 2**  
Physicochemical properties of defatted CNS starch.<sup>a</sup>

Starch source	Amylose content (wt.%)	Phosphate content (wt.%)	Swelling power (g/g)	Solubility content (wt.%)
Defatted CNS	24.65 ± 1.14	ND	5.12 ± 0.08	48.48 ± 0.43

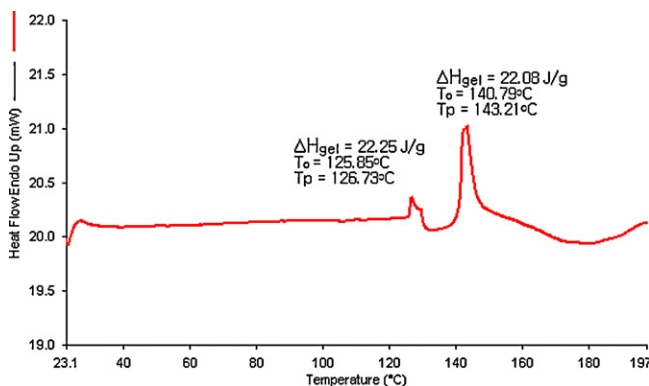
<sup>a</sup> Average of three independent experiments.

increase in transition temperature and enthalpy of gelatinization ( $\Delta H_{\text{gel}}$ ) (Barichello, Yada, Coffin, & Stanley, 1990).

Fig. 2 shows the heat flow profile of gelatinization process of the defatted CNS starch. There are two endothermic peaks at 123–133 °C and 135–179 °C which correspond to the occurrence of starch gelatinization. The first peak indicates the early gelatinization when hilum of starch granule started to swell and lost its crystalline state. As shown in the second peak, the gelatinization continued as the temperature was increased causing the collapse of crystallinity order within the starch granules which can be observed from the irreversible changes in properties such as starch swelling, pasting, loss of optical birefringence, uncoiling and dissociation of the double helices and starch solubility (Atwell, Hood, Lineback, Varriano-Morston, & Zohel, 1988; Hoover, 2001; Stevens & Elton, 1971). The onset temperature ( $T_o$ ) and peak gelatinization temperatures ( $T_p$ ) of defatted CNS starch were found to be 125.85 °C and 126.73 °C, respectively for initial gelatinization. The corresponding  $T_o$  and  $T_p$  for complete gelatinization of defatted CNS starch are 140.79 °C and 143.21 °C, respectively.

As shown in the thermograph (Fig. 2), the transition temperature and gelatinization enthalpy of defatted CNS starch were found to be 7.51 °C and 2.25 J/g, respectively for initial gelatinization. For complete gelatinization, the transition temperature and gelatinization enthalpy are 44.5 °C and 22.08 J/g, respectively. Compared to other commercial starches such as potato and corn starches reported by Singh et al. (2003), defatted CNS starch product has quite higher transition temperatures and enthalpy of gelatinization. This may be because the degree of crystallinity of defatted CNS starch was found to be higher than that of potato and corn starches. This result is in agreement with the high content of amylopectin (75.35 wt.%) found in defatted CNS starch.

Fig. 3 shows the heat flow profile of gelatinization process of the retrograded starch after storing the gelatinized starch for 7 days at 4 °C. The endothermic peak of the retrograded starch appeared between 54.21 and 145.72 °C. As observed from Fig. 3, retrogradation occurred at about 100.4 °C, lower than the gelatinization temperature which first occurred at 126.73 °C. Starch retrogradation enthalpy was found to be 13.12 J/g, 59.42% lower than that needed for gelatinization. Retrogradation happens due to molecular interactions (hydrogen bonding between starch chains)

**Fig. 2.** DSC curve of gelatinization of defatted CNS starch.  $\Delta H_{\text{gel}}$  = enthalpy of gelatinization,  $T_o$  = gelatinization onset temperature,  $T_p$  = gelatinization peak temperature.**Table 3**  
Thermal properties of defatted CNS starch.

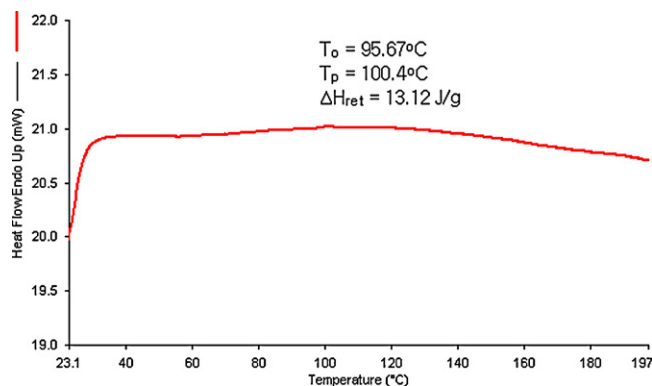
Thermal property	Defatted CNS starch
Gelatinization	
Onset temperature ( $T_o$ , °C)	125.85 <sup>a</sup> 140.79 <sup>b</sup>
Peak temperature ( $T_p$ , °C)	126.73 <sup>a</sup> 143.21 <sup>b</sup>
Temperature range (°C)	10 <sup>a</sup> 44 <sup>b</sup>
Enthalpy ( $\Delta H_{\text{gel}}$ , J/g)	2.25 <sup>a</sup> 22.08 <sup>b</sup>
Retrogradation	
Onset temperature ( $T_o$ , °C)	95.67
Peak temperature ( $T_p$ , °C)	100.4
Temperature range (°C)	91.51
Enthalpy ( $\Delta H_{\text{ret}}$ , J/g)	13.12
Decomposition	
Peak temperature (°C)	319

<sup>a</sup> Properties observed in early gelatinization.<sup>b</sup> Properties observed in complete gelatinization.

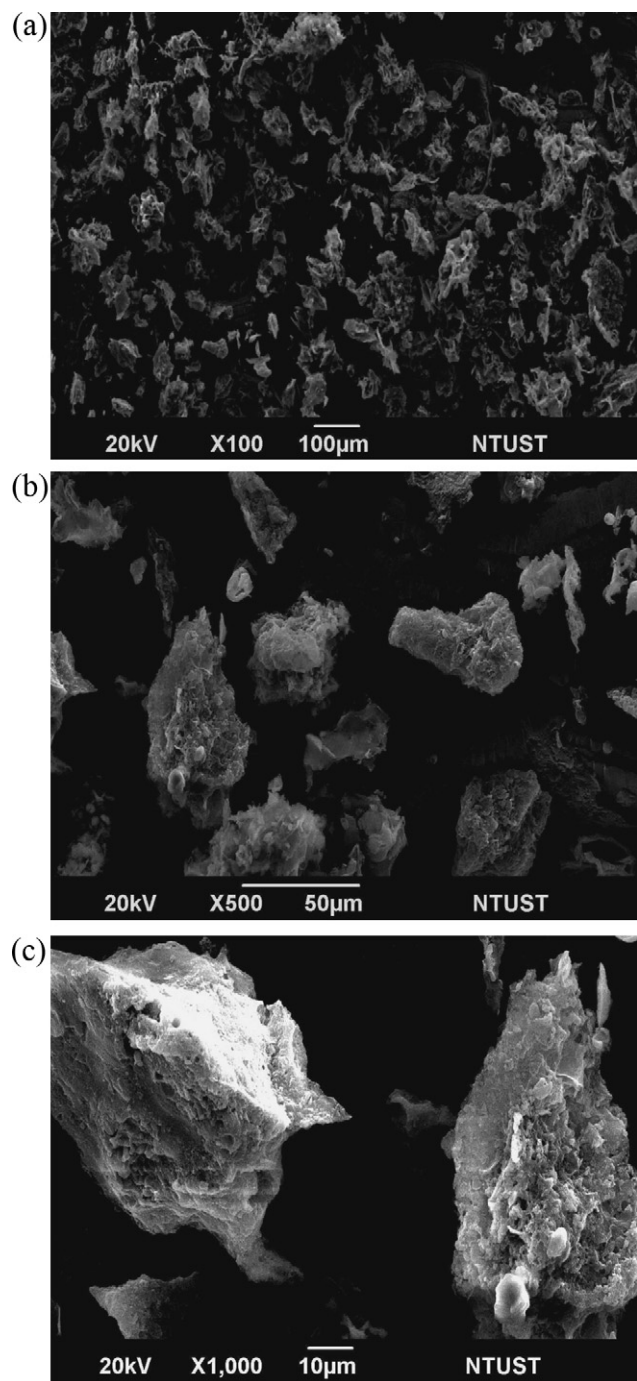
after cooling of the gelatinized starch paste, which results in the weaker starch crystallinity (Hoover, 2001; Sasaki, Yasui, & Matsuki, 2000). Ward, Hosene, and Seib (1994) also reported that recrystallization of amylopectin branch chains has been reported to occur in less ordered manner in stored starch gels than in native form. These reports explain the occurrence of lower retrogradation enthalpy and temperature range compared to that for gelatinization. The thermal properties studied are summarized in Table 3.

### 3.4. Morphological properties of starch

The scanning electron micrographs in Fig. 4 show the shape and size of the defatted CNS starch granules. Defatted CNS starch granules are irregular in shape with rough surfaces. Each granule also seems to have multiple layers and is fibrous. This unusual phenomenon is probably the result of the high concentration of resins contained in defatted CNS starch. Wang et al. (2003) studied starch resin and reported that starch granules were found to be irregular in shape, having multiple layers and rough surface when they

**Fig. 3.** DSC curve of retrogradation of defatted CNS starch.  $\Delta H_{\text{ret}}$  = enthalpy of retrogradation,  $T_o$  = retrogradation onset temperature,  $T_p$  = retrogradation peak temperature.





**Fig. 4.** SEM images of defatted CNS starch with (a) 100, (b) 500 and (c) 1000 magnification.

were bonded to polymer resins. CNS is a well-known material rich in polymeric materials. Tyman (1978) reported that cashew nut shell liquid possesses 20–24% polymeric material with more resins bonded to the CNS matrix. The roughness in the starch surface may also be caused by the use of alkali treatment during the purification of starch from defatted CNS. Defatted CNS starch granule size ranges from 25 to 50  $\mu\text{m}$  for small grains, 60 to 80  $\mu\text{m}$  for medium grains, and 100 to 140  $\mu\text{m}$  for big ones. Starch granule size is known to play an important role in affecting the physical properties of starch, especially its tensile strength. The tensile strength increases as the particle size of starch granule decreases. However, the effect of granule size on tensile strength decreases as resin film thickness on starch granule increases.

## 4. Conclusion

Starch was isolated from the defatted CNS using the wet-milling process. The product contains 85.01% starch, 0.93% protein, 3.74% fiber, 4.23% ash and 6.09% other impurities. The impurities contained in the starch were suspected to be polymeric resins, as observed from the morphological study of the starch granules. It was found that defatted CNS starch contains 24.65% amylose and 75.35% amylopectin. The starch grains are irregular in shape and have rough surfaces with sizes of 25–50  $\mu\text{m}$  for small grains, 60–80  $\mu\text{m}$  for medium grains, and 100–140  $\mu\text{m}$  for big grains. Two steps of gelatinization were observed during the analysis due to the presence of two endothermic peaks at 123–133  $^{\circ}\text{C}$  and 135–179  $^{\circ}\text{C}$  which correspond to the occurrence of early and complete starch gelatinization, respectively. The corresponding enthalpies of early and complete gelatinization were found to be 2.25 J/g and 22.08 J/g, respectively. The retrogradation of starch happens at 100.4  $^{\circ}\text{C}$  with enthalpy of 13.12 J/g, 59.42% lower than that for gelatinization and its major degradation occurs at 319  $^{\circ}\text{C}$ . Due to high crystallinity and the presence of bonded resins, defatted CNS starch maybe suitable as a potential renewable material for bioplastic industries. Moreover, because CNS is considered as in waste in the production of cashew nut, starch obtained from defatted CNS is considered to be a low-cost raw material and able to economically compete with other commercial sources. Further study on defatted CNS starch is still being done to determine its polymer behaviors.

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